ZooKeys 658: 63–80 (2017)
doi: 10.3897/zookeys.658.9738
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Two new species of *Tipula* (*Vestiplex*) from Southern China based on morphological and molecular data, with redescription of *Tipula* (*Vestiplex*) *bicalcarata* (Diptera, Tipulidae, Tipulinae)

Qiu-Lei Men[1], Chen W. Young[2], Pavel Starkevich[3], Yong-Fu Yu[4], Xiao-Ping Lei[5]

1 *School of Life Sciences, Province Key Laboratory of the Biodiversity Study and Ecology Conservation in Southwest Anhui; Research Center of Aquatic Organism, Conservation and Water Ecosystem Restoration in Anhui Province, Anqing Normal University, Anqing City 246011, Anhui, P. R. China* **2** *Section of Invertebrate Zoology, Carnegie Museum of Natural History, 4400 Forbes Avenue, Pittsburgh, Pennsylvania 15213-4080, USA* **3** *Nature Research Centre, Akademijos str. 2, LT-08412, Vilnius, Lithuania* **4** *Administrative Bureau of Leigongshan National Nature Reserve, Leishan County 557199, Guizhou, P. R. China* **5** *Administrative Bureau of Fanjingshan National Nature Reserve, Jiangkou County 554400, Guizhou, P. R. China*

Corresponding author: *Qiu-Lei Men* (menqiulei888@126.com)

Academic editor: *C. Borkent* | Received 1 July 2016 | Accepted 8 February 2017 | Published 23 February 2017

http://zoobank.org/5064C2E7-DE27-44DC-B520-167E941B7C10

Citation: Men Q-L, Young CW, Starkevich P, Yu Y-F, Lei X-P (2017) Two new species of *Tipula* (*Vestiplex*) from Southern China based on morphological and molecular data, with redescription of *Tipula* (*Vestiplex*) *bicalcarata* (Diptera, Tipulidae, Tipulinae). ZooKeys 658: 63–80. https://doi.org/10.3897/zookeys.658.9738

Abstract

Two new species of subgenus *Tipula* (*Vestiplex*) Bezzi, 1924, *T.* (*V.*) *leigongshanensis* Men & Young, **sp. n.** and *T.* (*V.*) *maoershanensis* Men & Young, **sp. n.** are described and illustrated. *Tipula* (*Vestiplex*) *bicalcarata* Savchenko, 1965 is redescribed and illustrated based on additional morphological characters. Partial mitochondrial cytochrome oxidase subunit I (COI) sequences of these three species are provided. Pairwise genetic distances among two new species and related species, *T.* (*V.*) *bicalcarata*, *T.* (*V.*) *coxitalis* Alexander, 1935, and *T.* (*V.*) *sternotuberculata* Alexander, 1935 range from 0.028 to 0.091 using Kimura-2-parameter model. Diagnostic features of the sperm pump for taxonomic use are discussed.

Keywords

China, COI, crane flies, Diptera, new species, sperm pump, Tipulidae, *Vestiplex*

Copyright *Qiu-Lei Men et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Tipula (*Vestiplex*) Bezzi, 1924 is a large subgenus in *Tipula* Linnaeus, 1758 with 170 species worldwide, distributed mainly in Oriental and Palaearctic regions (Oosterbroek 2016). *Tipula* (*Vestiplex*) was erected by Bezzi (1924) with the type species *Tipula cisalpina* Riedel, 1913 from the West Palaearctic region by original designation. China hosts 67 species of this subgenus, distributed mainly in the southern part of the country (Oosterbroek 2016). It is characterized by the following characters: antennae short to elongate, with flagellum very strongly incised in male; thorax with prescutum generally glabrous; tibial spur with formulation 1-2-2; squama naked; R_{1+2} entire, Rs one-half longer than m-cu; male hypopygium generally very sclerotized and blackened, with ninth tergite forming a saucer and produced into a pair of acute projections, gonocoxite generally elongated; female ovipositor with cerci heavily sclerotized and equipped with saw-like teeth on the lower margins, but smooth in some species; hypovalvae small, extending scarcely beyond the base of cerci (Alexander 1935a).

Many species of subgenus *Tipula* (*Vestiplex*) were originally placed in the subgenus *Oreomyza* Pokorny, 1887, which was subsequently treated as a synonym of the subgenus *Tipula* (*Pterelachisus*) Rondani, 1842. The subgeneric status of species in these two subgenera has always been a troublesome issue to crane fly researchers when identifying from morphological characters only. Therefore, molecular characters have become an important addition to morphological characters, and have been proven successful for separating and identifying insect species when applied to the following instances: fragmented specimens, closely related species with extremely similar morphology, cryptic species, dubious correspondence between larva and adult, or male and female (Hebert et al. 2004, Barcenas et al. 2005, Johanson 2007, Men and Qin 2011, Yang et al. 2012, Serjeant and Beebee 2013), as well as defining the taxonomic status of taxa (Lim et al. 2013, Reijnen et al. 2014).

Two new species of the subgenus *Tipula* (*Vestiplex*) were noticed among recently collected specimens from Guangxi Zhuang Autonomous Region and Guizhou Province in southern China. The present paper provides the descriptions and illustrations of the external morphology of the new species. The COI sequences of the new species are also provided in order to augment characteristic data. The COI sequence data were used to calculate the pairwise genetic distances among the new species and related species, to delineate and establish the two new species. And finally, the subgeneric position of new species is argued based on COI sequences of the new species and some known species of subgenera *Tipula* (*Vestiplex*) and *Tipula* (*Pterelachisus*). *Tipula* (*V.*) *bicalcarata* is redescribed and illustrated based on additional morphological characters. New distribution records for *T.* (*V.*) *bicalcarata* are provided. Diagnostic features and use of the sperm pump for taxonomy are discussed.

Material and methods

Taxonomic analysis

Photographs of the body parts of male adults were obtained using a SOIFXTZ-E stereomicroscope (SOIF, Shanghai, China). The hypopygium of each male was removed and macerated in 10% NaOH for one hour in a 50°C water bath, observed in glycerin and illustrated under a SOIFXTZ-E stereomicroscope (SOIF, Shanghai, China). The body length was measured from the vertex of head to the tip of hypopygium. All measurements were made in millimeters (mm) with the aid of a digital caliper. The angles between compressor apodemes and posterior immovable apodemes of the sperm pump were measured by ImageJ software. The terminology and methods of description and illustration followed that of Alexander and Byers (1981) and Frommer (1963). The type specimens are deposited in the animal specimen room, School of Life Sciences, Anqing Normal University, Anhui Province, P. R. China. Qiu-Lei Men and Chen W. Young were responsible for the taxonomic portion of this paper, thus are the authors of the new species.

Molecular analysis

Genomic DNA was extracted from one leg of dry preserved specimen using Biomiga Insect gDNA Kit (Biomiga, USA). Genomic DNA of four type specimens of new species and two specimens of *T.* (*V.*) *bicalcarata* was extracted. The partial sequence of the mitochondrial COI gene was amplified using the universal primers for metazoan invertebrates, LCO1490 (5'-GGTCAACAAATCATAAAGATATTG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAAT-3') (Folmer et al. 1994). PCR amplifications were employed using a final volume of 20 μl containing 10 μl 2 × Pfu PCR MasterMix (Tiangen, Beijing, China), 0.75 μl each primer (10 μM), 1 μl DNA template and 7.5μl ddH_2O. PCR amplification was employed with denaturation at 95 °C for 5 mins, followed by 45 cycles of 30 s at 95 °C for denaturation, 30 s at 50 °C for annealing and 1 min 30 s at 72 °C for extension, with a final extension at 72 °C for 10 mins. All PCR sets included a negative control reaction tube in which all reagents were included but the template DNA. After electrophoresis with 1% agarose gel, the target DNA was sent to Genescript Biotechnology Co., Ltd. (Nanjing, China) for sequencing. The partial COI sequences were aligned with CLUSTAL X (Thompson et al. 1997). The aligned sequences were processed by MEGA 6.0 (Tamura et al. 2013) for analyzing the DNA sequence compositions and calculating pairwise genetic distance based on the Kimura-2-parameter model (Kimura 1980). Partial COI sequences of new species and *T.* (*V.*) *bicalcarata* obtained in this study were submitted to GenBank with the following accession numbers: *T.* (*V.*) *bicalcarata* (KU844262), *T.* (*V.*) *leigongshanensis* sp. n. (KU844261) and *T.* (*V.*) *maoershanensis* sp. n. (KU844263). For revealing the subgeneric position of new species, a maximum likelihood tree was constructed using MEGA 6.0 with 1000

Table 1. Accession numbers and sources of COI sequences of some known species in *Tipula* (*Pterela-.(chisus*) and *Tipula* (*Vestiplex*

Species	Accession numbers	Sources
Tipula (*Pterelachisus*) *stenostyla*	JQ912049	Pilipenko et al. 2012
Tipula (*Pterelachisus*) *winthemi*	JQ912057	Pilipenko et al. 2012
Tipula (*Pterelachisus*) *jutlandica*	JQ912035	Pilipenko et al. 2012
Tipula (*Pterelachisus*) *octomaculata*	JQ912044	Pilipenko et al. 2012
Tipula (*Pterelachisus*) *submarmorata*	JQ912050	Pilipenko et al. 2012
Tipula (*Pterelachisus*) *pseudovariipennis*	JQ912047	Pilipenko et al. 2012
Tipula (*Pterelachisus*) *varipennis*	JQ912054	Pilipenko et al. 2012
Tipula (*Pterelachisus*) *mutila*	JQ912042	Pilipenko et al. 2012
Tipula (*Pterelachisus*) *wahlgreni*	JQ912055	Pilipenko et al. 2012
Tipula (*Pterelachisus*) *truncorum*	JQ912051	Pilipenko et al. 2012
Tipula (*Vestiplex*) *arctica*	KU374459	Wirta et al. 2016
Tipula (*Vestiplex*) *canadensis*	KM571431	Barcoding Canada Data Release
Tipula (*Vestiplex*) *bicalcarata*	KU844262	New submission in present study
Tipula (*Vestiplex*) *coxitalis*	Not released	Provided by second author
Tipula (*Vestiplex*) *sternotuberculata*	Not released	Provided by second author

bootstraps (Tamura et al. 2013) based on COI sequences of the new species and known species of *Tipula* (*Vestiplex*) and *Tipula* (*Pterelachisus*), which were mainly downloaded from GenBank (accession numbers presented in Table 1).

Results

COI sequences analysis

Sequences containing 623 base pairs were recovered for the studied species, which included 75 variable sites and 548 conserved sites. Variable sites are shown in Figure 38. Variable sites included 25 parsimony informative sites and 50 singleton sites. The pairwise genetic distances between the two new species and related species ranged from 0.028 to 0.091 based on Kimura-2-parameter model (Table 2).

The maximum likelihood tree (Fig. 39) showed that the two new species clustered with species of *Tipula* (*Vestiplex*), suggesting their subgeneric position.

Taxonomy

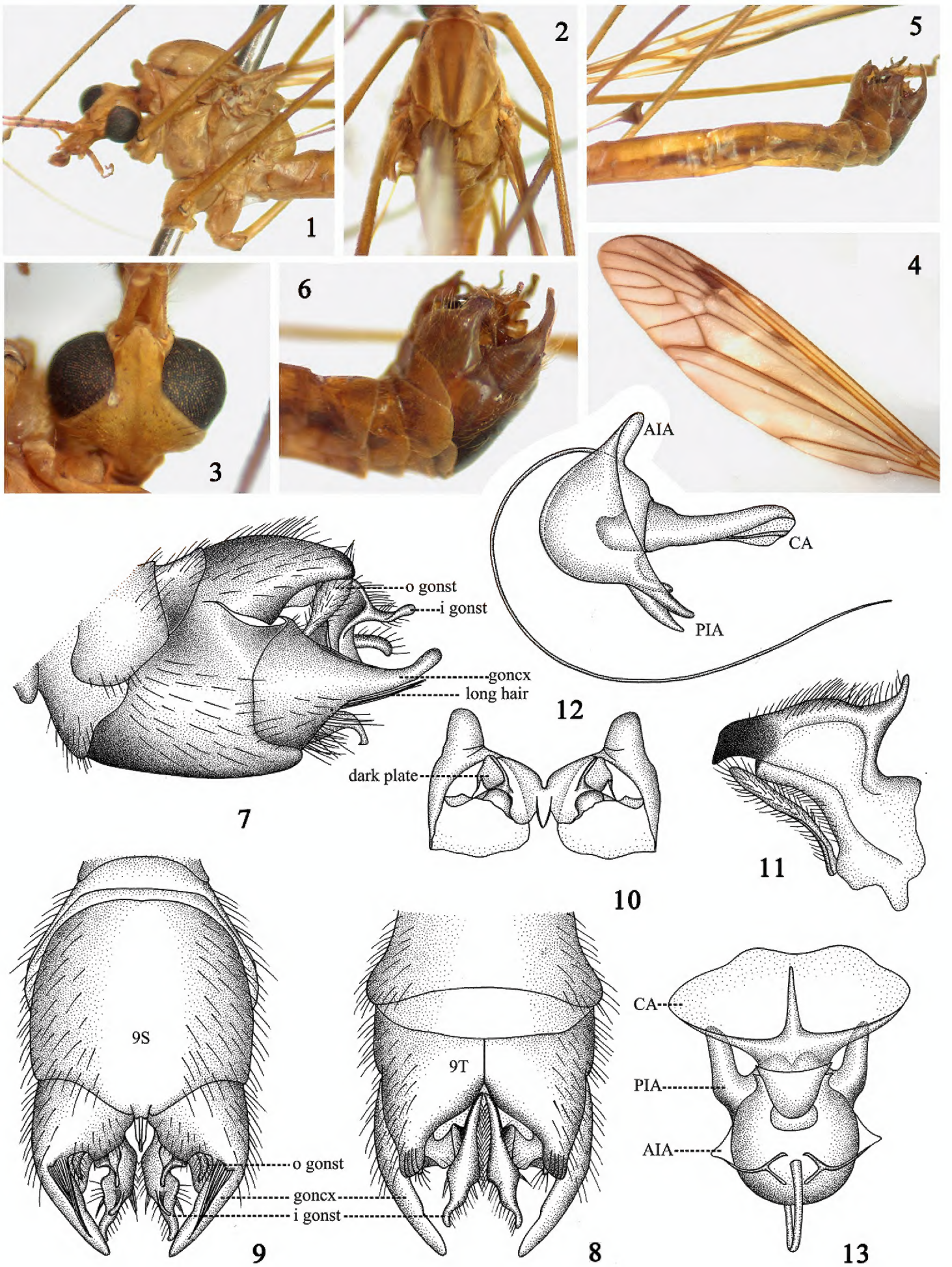
Tipula (*Vestiplex*) *bicalcarata* Savchenko, 1965

Diagnosis. Body generally reddish brown in color (Figs 1–6). Ninth tergite separated by a deeply V-shaped notch, lateral angles produced into an ear-like lobe (Fig. 8).

Table 2. Pairwise genetic distances for COI gene sequences of species examined in the present study.

Species	*lei*	*ste-t*	*cox*	*bic*	*mao*	*win*	*wah*	*var*	*sub*	*ste-s*	*pse*	*oct*	*mut*	*jut*	*arc*	*can*
ste-t	0.086															
cox	0.042	0.084														
bic	0.059	0.088	0.057													
mao	0.047	0.091	0.040	0.028												
win	0.117	0.126	0.119	0.105	0.109											
wah	0.138	0.139	0.136	0.128	0.132	0.067										
var	0.126	0.132	0.124	0.118	0.118	0.057	0.077									
sub	0.138	0.141	0.134	0.130	0.132	0.062	0.089	0.034								
ste-s	0.132	0.128	0.132	0.111	0.118	0.038	0.084	0.079	0.072							
pse	0.138	0.134	0.130	0.130	0.130	0.069	0.085	0.019	0.041	0.088						
oct	0.134	0.134	0.136	0.121	0.119	0.054	0.086	0.080	0.084	0.064	0.082					
mut	0.134	0.134	0.140	0.128	0.138	0.075	0.028	0.085	0.089	0.082	0.094	0.087				
jut	0.128	0.132	0.124	0.117	0.117	0.039	0.074	0.077	0.081	0.054	0.077	0.066	0.081			
arc	0.132	0.136	0.128	0.128	0.132	0.137	0.138	0.142	0.148	0.140	0.148	0.154	0.144	0.150		
can	0.136	0.136	0.134	0.127	0.130	0.121	0.140	0.144	0.150	0.122	0.142	0.145	0.129	0.133	0.088	

Abbreviation: *lei*, *T.* (*V.*) *leigongshanensis*; *ste-t*, *T.* (*V.*) *sternotuberculata*; *cox*, *T.* (*V.*) *coxitalis*; *bic*, *T.* (*V.*) *bicalcarata*; *mao*, *T.* (*V.*) *maoershanensis*; *win*, *T.* (*P.*) *winthemi*; *wah*, *T.* (*P.*) *wahlgreni*; *var*, *T.* (*P.*) *varipennis*; *sub*, *T.* (*P.*) *submarmorata*; *ste-s*, *T.* (*P.*) *stenostyla*; *pse*, *T.* (*P.*) *pseudovariipennis*; *oct*, *T.* (*P.*) *octomaculata*; *mut*, *T.* (*P.*) *mutila*; *jut*, *T.* (*P.*) *jutlandica*; *arc*, *T.* (*P.*) *arctica*; *can*, *T.* (*P.*) *canadensis*.



Figures 1–13. *Tipula* (*Vestiplex*) *bicalcarata* **1** thorax, lateral view **2** thorax, dorsal view **3** head, dorsal view **4** wing **5** abdomen and hypopygium, lateral view **6** hypopygium, lateral view **7** hypopygium, lateral view **8** hypopygium, dorsal view **9** hypopygium, ventral view **10** tergite nine, ventral view **11** inner gonostylus and outer gonostylus **12** sperm pump, lateral view **13** sperm pump, dorsal view. Abbreviation: AIA, anterior immovable apodeme; CA, compressor apodeme; goncx, gonocoxite; i gonst, inner gonostylus; o gonst, outer gonostylus; PIA, posterior immovable apodeme.

Gonocoxite extended into a long arm, directed caudally, widest at base and narrowed to apex (Figs 6–7).

Redescription. Male. Length: *Body*: 12.5–13.0 mm (excluding antenna, n = 5); *Wing*: 19.0–19.5 mm (n = 5); *Antenna*: 4.3–4.5 mm (n = 5).

Head. Reddish-brown except as noted. Vertex without marking (Fig. 3). Antenna reddish-brown, 13-segmented, bent backward extending to root of halteres; scape cylindrical, expanded apically; pedicel short, hemispherical; each flagellomere subequal in length, basal enlargement black with abundant black verticils, longest ones subequal to length of corresponding flagellomeres. Palpi with basal three segments reddish-brown, last one black.

Thorax. Generally reddish-brown except as noted. Pronotum light yellow laterally, gradually becoming reddish-brown, black medially (Figs 1–2). Prescutum with three light brown stripes, median one marginally suffused with brown at basal half, humeral angle with a black marking at lateral side (Figs 1–2). Scutum with two light brown markings (Fig. 2). Scutellum with dark median stripe. Postnotum wholly reddish-brown. Pleura entirely reddish brown (Fig. 1). Legs slender, coxae, trochanters and femora reddish-brown, tibiae and tarsi light brown. Halteres stem reddish-brown, knob darker. Wings reddish-brown, cells c and sc darker than ground color; stigma dark brown; Rs suffused with dark brown at origin point; discal cell transparent; some large hyaline areas at cells m and a (Fig. 4). Venation: R_{1+2} entire, discal cell narrow, elongated, petiole of cell m_1 distinctly shorter than discal cell (Fig. 4).

Abdomen. Generally reddish brown except as noted. Abdominal tergites with brown lateral stripes (Fig. 5). Hypopygium brown (Fig. 6). Tergite nine with a deeply V-shaped notch, separated medially into two parts, produced into ear-like lobe in lateral angle, beneath it with a dark plate on ventral side (Figs 8, 10). Sternite nine broad, not fused with tergite nine (Figs 6–7, 9). Gonocoxite extended into a long arm, directed caudally, widest at base, tapering to apex, ventrally of gonocoxite with a central band of long black setae (Figs 5–9). Outer gonostylus elongated, thin, generally curled as a tube (Fig. 11). Inner gonostylus tapering to apex, terminating in a black beak, with horn-shaped process on its dorsal side (Fig. 11).

Sperm pump. Compressor apodeme fan-shaped with two rounded extensions marginally (Fig. 11), forming a 45° angle with posterior immovable apodeme (Fig. 12). Posterior immovable apodeme distinctly shorter than compressor apodeme, gradually narrowed to apex (Fig. 12). Anterior immovable apodeme short, gradually narrowed to apex (Fig. 12). Aedeagus tubular, almost 3.0 times longer than sperm pump, acute apically (Fig. 12).

Examined material. 3 males, Guizhou Province, Fanjingshan Mountain, 27°55'N, 108°38'E, 12 June 2015, Guoxi Xue leg. 2 males, Guangxi Zhuang Autonomous Region, Dayaoshan Mountain, 24°08'N, 110°11'E, 14 May 2016.

Distribution. China (Beibei, Chongqing; new distribution records in China: Fanjingshan Mountain, Guizhou Province; Dayaoshan Mountain, Guangxi Zhuang Autonomous Region, see Fig. 40).

***Tipula* (*Vestiplex*) *leigongshanensis* Men & Young, sp. n.**
http://zoobank.org/46AEB6CF-724D-4965-A698-A3B43E822DC6

Diagnosis. Body generally brown in coloration (Figs 14–19). Hypopygium expanded and black (Fig. 19). Sternite nine with a pair of nail-shaped processes (Figs 19–20). Gonocoxite produced into a bird-head-shaped lobe (Figs 19–20).

Description. Male. Length: *Body*: 11.5–12.0 mm (excluding antenna, n = 4); *Wing*: 16.0–16.5 mm (n = 4); *Antenna*: 4.5–4.7 mm (n = 4).

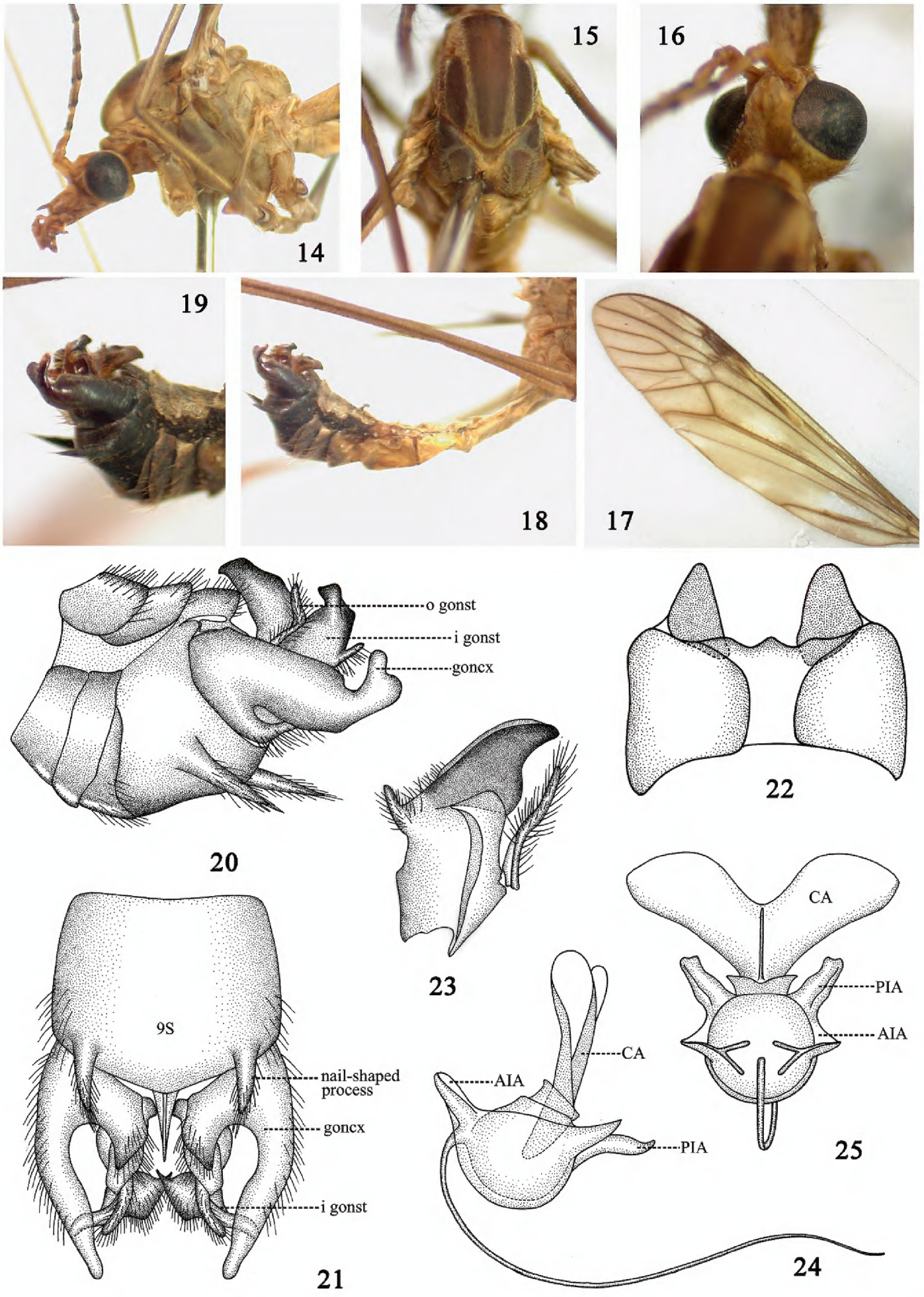
Head. Generally reddish brown except as noted. Rostrum light brown with brownish nasus (Fig. 14). Antenna 13-segmented, bent backward extending to root of first abdominal segment; scape reddish-brown, cylindrical, expanded apically; pedicel reddish-brown, short; flagellum brown, flagellomere subequal in length, basal enlargement black with abundant black verticils, longest ones subequal to length of corresponding flagellomeres. Palpi entirely reddish brown. Vertex without marking (Fig. 16).

Thorax. Generally brown except as noted. Pronotum brown, becoming black medially. Prescutum with three brown stripes. Scutum with two dark brown markings (Fig. 15). Scutellum with a dark median stripe (Fig. 15). Postnotum entirely brown. Pleura reddish-brown, tinged with brown at anepimeron and anepisternum (Fig. 14). Legs slender, coxae and trochanters brown, femora brown with tip black, tibiae, and tarsi black. Halteres with stem brown, knob darker. Wings reddish-brown, cells c and sc darker than ground color; stigma dark brown; Rs suffused with dark brown at origin point; discal cell transparent; several large hyaline areas at cells r, m and a (Fig. 17). Venation: R_{1+2} entire, discal cell narrow, elongated, petiole of cell m_1 distinctly shorter than discal cell (Fig. 17).

Abdomen with basal four segments brown, remaining segments generally darker, with black distinct median and lateral stripes, sternites entirely reddish-brown (Fig. 18). Hypopygium black (Fig. 19). Tergite nine separated medially into two parts, connected with membranous extension, hind margin of tergite nine forming W-shaped emargination (Fig. 22). Ventrad of tergite nine with two semi-triangular process (Fig. 22). Sternite nine broad, not fused with tergite nine, with a pair of nail-shaped processes arising from lateral sides, caudally directed, densely covered with long black setae (Figs 19–21). Gonocoxite produced into bird-head-shaped lobe, with small light-colored depression on base (Figs 19–20). Outer gonostylus elongated, thin, generally curled as a tube (Fig. 23). Inner gonostylus produced into black beak, with horn-shaped process on its dorsal side (Fig. 23).

Sperm pump with compressor apodeme V-shaped, forming a 55° angle with posterior immovable apodeme (Figs 24–25). Posterior immovable apodeme distinctly shorter than compressor apodeme, gradually narrowed to apex (Fig. 24). Anterior immovable apodeme short, gradually narrowed to apex (Fig. 24). Aedeagus tubular, almost 2.5 times longer than sperm pump, acute apically (Fig. 24).

Type material. Holotype male. **CHINA:** Guizhou Province, Leigongshan Mountain, 26°21'N, 108°13'E, 2 June 2015, Guoxi Xue leg. **Paratype.** 1 male, same data as holotype. 2 males, Guizhou Province, Leigongshan Mountain, 26°21'N, 108°13'E, 14 May 2016, Qiulei Men leg.



Figures 14–25. *Tipula* (*Vestiplex*) *leigongshanensis*, sp. n. **14** thorax, lateral view **15** thorax, dorsal view **16** head, dorsal view **17** wing **18** abdomen and hypopygium, lateral view **19** hypopygium, lateral view **20** hypopygium, lateral view **21** hypopygium, ventral view **22** tergite nine, dorsal view **23** inner gonostylus and outer gonostylus **24** sperm pump, lateral view **25** sperm pump, dorsal view. Abbreviation: AIA, anterior immovable apodeme; CA, compressor apodeme; goncx, gonocoxite; i gonst, inner gonostylus; o gonst, outer gonostylus; PIA, posterior immovable apodeme.

Distribution. China (Leigongshan Mountain, Guizhou Province, Fig. 40).

Remarks. The new species is placed in subgenus *Tipula* (*Vestiplex*) because of its male hypopygium with elongated gonocoxite, which is also supported by the results of the molecular analysis (Fig. 39). The new species is mostly similar to *T.* (*V.*) *sternotuberculata* Alexander, 1935, from Taiwan, China, in the body color and the structure of hypopygium. *Tipula* (*V.*) *leigongshanensis* can be easily distinguished from the latter by the bird-headed distal end of gonocoxite (distal end of gonocoxite roundly expanded in *T.* (*V.*) *sternotuberculata* as figure 32 in Alexander, 1935b), and the nail-shaped process on sternite nine distinctly thinner than that of *T.* (*V.*) *sternotuberculata*. Moreover, pairwise genetic distance between these two species is 0.086 based on the Kimura-2-parameter model (the COI sequence of *T.* (*V.*) *sternotuberculata* is unpublished data, provided by the second author). Of 40 interspecific comparisons of genetic distance values among known species, 33 are equal to or lower than 0.086, which could suggest significant genetic variation between the new species and *T.* (*V.*) *sternotuberculata* (Table 2).

Etymology. The specific epithet is a noun '*leigongshan*' with Latin suffix '*ensis*', referring to the type locality of the new species.

Tipula (*Vestiplex*) *maoershanensis* Men & Young, sp. n.

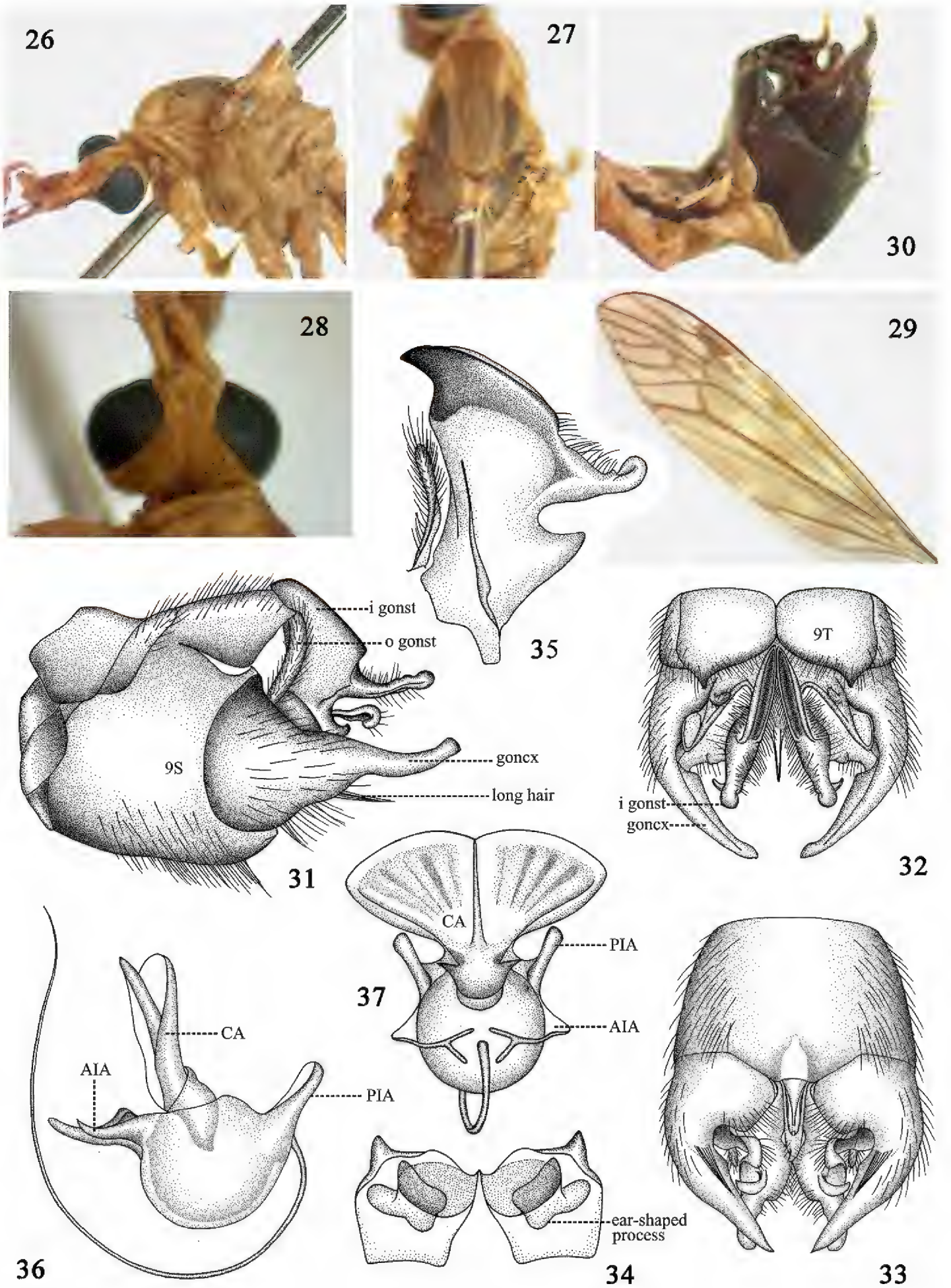
http://zoobank.org/9E2254C1-0F95-4CBA-994E-2649C913FF72

Diagnosis. Whole body generally brownish in coloration (Figs 26–29). Prescutum with median stripe brighter than lateral stripes (Fig. 27). Hypopygium dark brown (Fig. 30). Gonocoxite produced into a long arm, basally broad and gradually narrowed to the apex (Figs 30–31).

Description. Male. Length: *Body*: 12.0–12.3 mm (excluding antenna, n = 2); *Wing*: 16.2–16.5 mm (n = 2); *Antenna*: 3.5–3.7 mm (n = 2).

Head. Rostrum light brown with brownish nasus (Fig. 26). Antenna 13-segmented, bent backward extending to root of wing; scape reddish brown, cylindrical, expanded apically; pedicel reddish-brown, short; flagellum brown, flagellomere subequal in length, basal enlargement black with abundant black verticils, longest ones subequal to length of corresponding flagellomeres. Palpi entirely reddish brown. Vertex without marking (Fig. 28).

Thorax. Generally brown except as noted. Pronotum brown, changed to black medially. Prescutum with three brown stripes, median one darker than laterals (Fig. 27). Scutum with two dark brown markings (Fig. 27). Scutellum with dark median stripe. Postnotum wholly brown. Pleura entirely reddish-brown (Fig. 26). Legs slender, coxae and trochanters brown, femora brown with tip black, tibiae brown with tip black, tarsi black. Halteres with stem brown, knob darker. Wings reddish-brown, cells c and sc darker than ground color; stigma dark brown; Rs suffused with dark brown at origin point; discal cell transparent; several large hyaline areas at cells r, m and a (Fig. 29). Venation: R_{1+2} entire, discal cell narrow, elongated, petiole of cell m_1 distinctly shorter than discal cell (Fig. 29).



Figures 26–37. *Tipula* (*Vestiplex*) *maoershanensis*, sp. n. **26** thorax, lateral view **27** thorax, dorsal view **28** head, dorsal view **29** wing **30** hypopygium, lateral view **31** hypopygium, lateral view **32** hypopygium, dorsal view **33** hypopygium, ventral view **34** tergite nine, ventral view **35** inner gonostylus and outer gonostylus **36** sperm pump, lateral view **37** sperm pump, dorsal view. Abbreviation: AIA, anterior immovable apodeme; CA, compressor apodeme; goncx, gonocoxite; i gonst, inner gonostylus; o gonst, outer gonostylus; PIA, posterior immovable apodeme.

```
                    1111112222222222222223333333333333333344444444444455555555555566
                    1124456674667890012334445677880011233345567778890122235667789112277788999 01
                    2322816954891051462471358469283609703691440581462406973231793165803925147 95
leigongshanensis    ATTAAATTTATCGTCTTGTCCCGTTTCATTCCTGCCTTCATTTAATTAATATGGTCCTAGATGTATTTTTAATTC
sternotuberculata   T.CT.T.C.T.TAG.AAACT.TAC..TGCA.TC.TTAGT.A.CTTCC.T.CCA.CT.CTT.CA.TACCCC.....
coxitalis           .C..GG...TC.AAT..A...TAC......T.C....A.G...G........A.C....AG.T.....C...CC.
bicalcarata         .....GC.CT.TA....A.TTT.CCC.G..T.CAT..A...C...C.T.C..A..TT..T..AA...C..GGCCT
maoershanensis      ....GG..CT.TA....A.TTT.C.C....T......AT..C.....G.C..AC..T..AG.AA...C....CC.
```

Figure 38. Variable sites from mitochondrial COI gene sequence alignment. Numbers arrayed vertically represent the positions of the nucleotides.

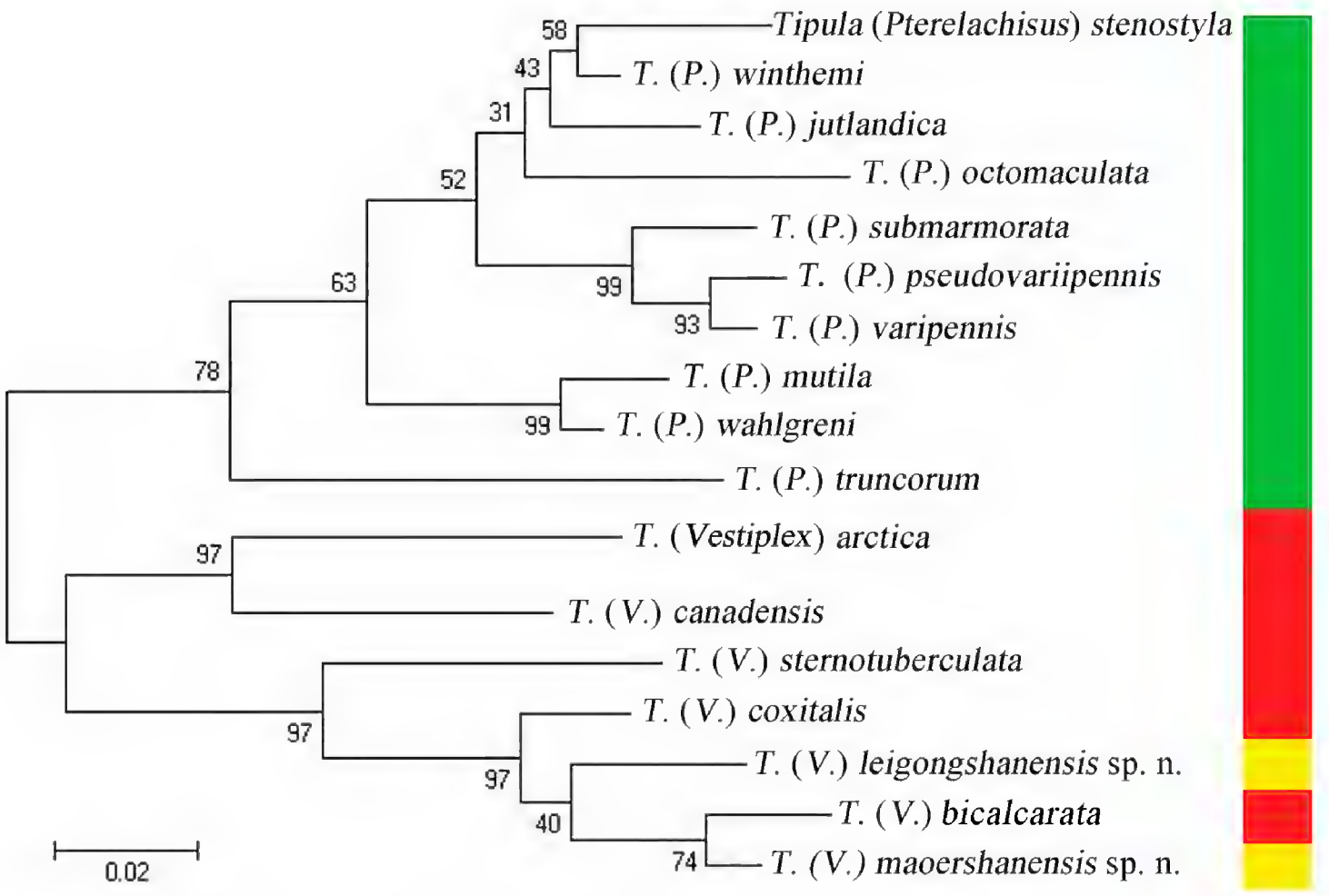


Figure 39. Maximum likelihood tree based on COI sequences of new species and some known species of *Tipula* (*Vestiplex*) and *Tipula* (*Pterelachisus*). Green region represents species of *Tipula* (*Pterelachisus*); red region represents species of *Tipula* (*Vestiplex*); yellow region represents new species.

Abdomen. Abdominal tergites reddish-brown with brown lateral stripes, sternites entirely reddish brown. Hypopygium dark brown (Fig. 30). Tergite nine entirely divided into two parts, produced into horn-shaped process in the lateral angle, ventral side of tergite nine with a pair of ear-shaped processes (Figs 32, 34). Gonocoxite extended into long arm, directed caudally, widened at base and narrowed to apex; ventrally, with central band of long black setae (Figs 30–31, 33). Outer gonostylus elongated, thin, generally curled as a tube (Fig. 35). Inner gonostylus produced into black beak, with a finger-shaped, rounded, expanded apically process on dorsal side.

Sperm pump with compressor apodeme fan-shaped, shallowly emarginated in middle, deeper coloration in median region, suffused by several dark stripes, forming a 65° angle with posterior immovable apodeme (Fig. 37). Posterior immovable apodeme

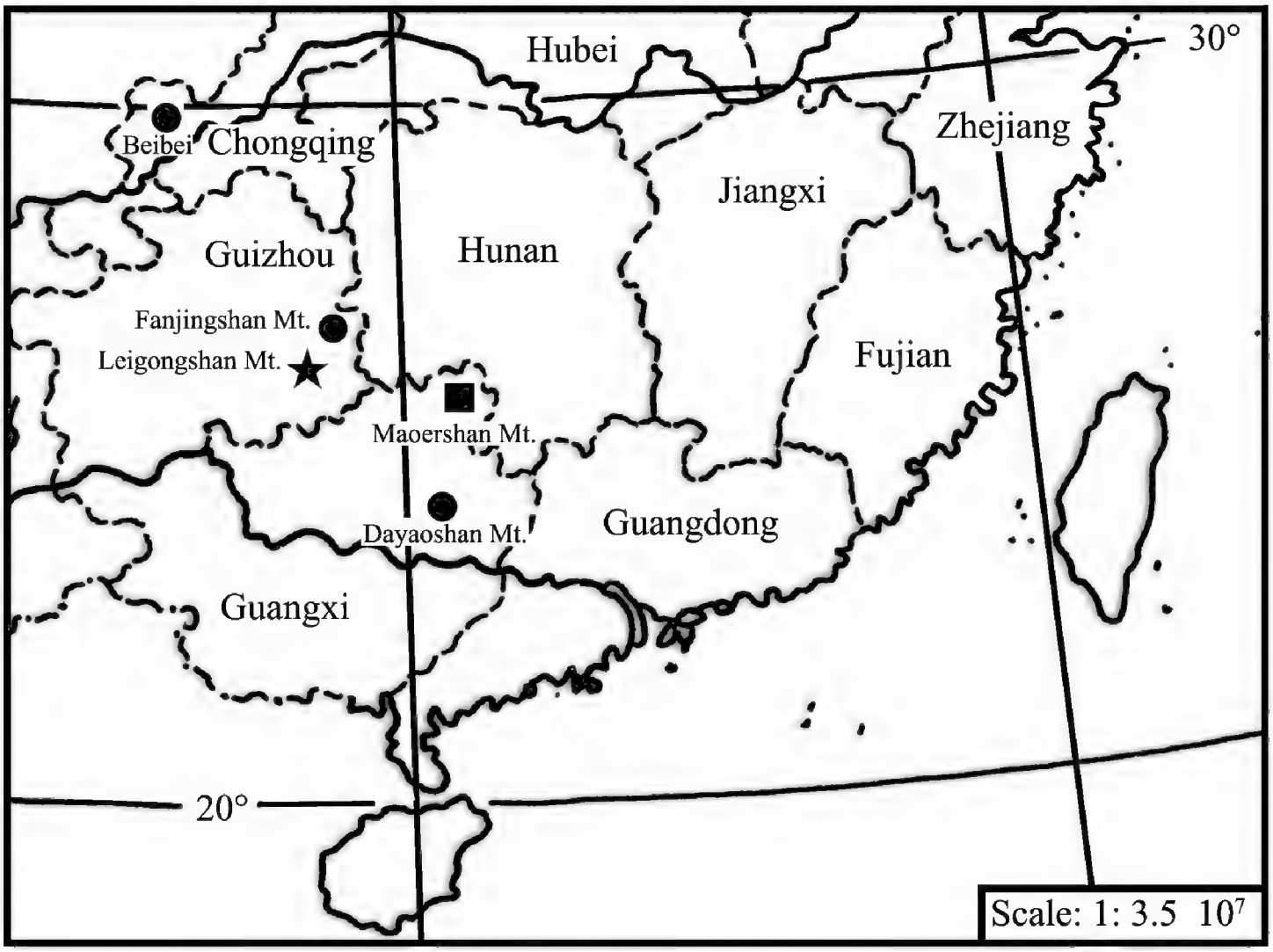


Figure 40. Geographic distribution of the species examined: *T.* (*V.*) *bicalcarata* (●), *T.* (*V.*) *leigongshanensis* sp. n. (★), *T.* (*V.*) *maoershanensis* sp. n. (■).

distinctly shorter than compressor apodeme, gradually narrowed to apex (Fig. 36). Anterior immovable apodeme short, gradually narrowed to apex (Fig. 36). Aedeagus tubular, almost 2.5 times longer than sperm pump, acute apically (Fig. 36).

Type material. Holotype male. **CHINA:** Guangxi Zhuang Autonomous Region, Maoershan Mountain, 25°48'N, 110°25'E, 21 May 2015, Guoxi Xue leg. **Paratype.** 1 male, same data as holotype.

Distribution. China (Maoershan Mountain, Guangxi Zhuang Autonomous Region, Fig. 40).

Remarks. The new species is placed in subgenus *Tipula* (*Vestiplex*) because of its male hypopygium with elongated gonocoxite, which is also supported by the molecular analysis (Fig. 39). The new species is similar to *Tipula* (*Vestiplex*) *coxitalis* Alexander, 1935, in the body color and the structure of gonostylus, it can be easily distinguished from the latter by the gonocoxite not expanded distally (distal end of gonocoxite expanded in *T.* (*V.*) *coxitalis* figs 30, 31 in Alexander, 1935b), by the sternite without a band of long setae on each lateral sides (with such long hair in *T.* (*V.*) *coxitalis* fig. 30 in Alexander, 1935b), by the inner gonostylus with dorsal process distinctly larger than that of *T.* (*V.*) *coxitalis*. Pairwise genetic distance between *T.* (*V.*) *maoershanensis* sp. n. and *T.* (*V.*) *coxitalis* is 0.048 based on the Kimura-2-parameter model, suggesting distinct genetic variation between these two species. Six lower values of pairwise genetic

distance were observed in comparisons to known species (Table 2), which could be taken as indirect evidence. The new species is also similar to *T.* (*V.*) *bicalcarata* by the body color and the structure of gonocoxite. It can be easily distinguished from the latter by the tergite nine produced into a horn-like process in lateral angle (the tergite nine produced into an ear-shaped process in *T.* (*V.*) *bicalcarata*, Fig. 8), the dorsal angle of inner gonostylus rounded and expanded apically (the dorsal angle of inner gonostylus gradually narrowed to apex in *T.* (*V.*) *bicalcarata*, Fig. 11). Pairwise genetic distance between these two species is 0.028 based on the Kimura-2-parameter model. Although the value is relatively low, it is equal to the comparison value of *T.* (*P.*) *wahlgreni* and *T.* (*P.*) *mutila*, while higher than the comparison value of *T.* (*P.*) *pseudovariipennis* and *T.* (*P.*) *varipennis* (0.019).

Etymology. The specific epithet is a noun '*maoershan*' with Latin suffix '*ensis*', referring to the distribution of the new species.

Sperm pump

The structures of sperm pumps in the two new species and *T.* (*V.*) *bicalcarata* showed substantial variation in shapes and colors, especially the shapes of the compressor apodemes, which suggests that the characters of sperm pumps can be used to distinguish closely related species (Table 3).

Discussion

In most insect orders, males transfer their sperm to females by spermatophore (Khalifa 1949, Hünefeld and Beutel 2005). However, in most families of Diptera, sperm is transferred by means of semen pump or sperm pump, which also occurs in other members of Antliophora including Mecoptera and Siphonaptera (Hünefeld and Beutel 2005). The sperm pump of crane-flies shows rich morphological diversity in different taxa. sperm pumps have been successfully demonstrated to separate related species within the subgenus *Tipula* (*Yamatotipula*) and the genus *Nephrotoma,* based on the angle between the posterior immovable apodeme and the compressor apodeme, the length of posterior immovable apodeme, and the shape and color of compressor apodeme in previous studies (Men et al. 2015a, 2015b). Frommer (1963) divided the sperm pump of crane-flies into three types based on anatomical studies of male reproductive systems of tipuline crane-flies species from North American. In the present study, sperm pumps of new species belong to type III, which is the most common type characterized by the strongly bowed intromittent organ. After comparing the structures of sperm pumps in the three species examined in this present study, it was observed that they showed substantial variation in shapes of compressor apodemes and color, which indicates that the characters of sperm pumps are useful in distinguishing closely related species.

Table 3. Characters of sperm pump in three species of *Tipula* (*Vestiplex*).

Species	Compressor apodeme (CA)	Posterior immovable apodeme (PIA)	Anterior immovable apodeme (AIA)
bicalcarata (Figs 12, 13)	Fan-shaped, marginally with two rounded extensions. Generally reddish-brown.	Distinctly shorter than CA, narrow, acute apically, in a 45° angle with CA.	Small, inner lateral margins expended in lateral view, not connected apically.
leigongshanensis (Figs 24, 25)	Y-shaped. Generally reddish-brown.	Distinctly shorter than CA, narrow, acute apically, in a 55° angle with CA.	Gradually narrowed to apex, not connected apically. Small, inner lateral margins expended in lateral view.
maoershanensis (Figs 36, 37)	Fan-shaped, shallowly emarginated medially, deeper in coloration in median region, suffused by several dark stripes.	Distinctly shorter than CA, narrow, acute apically, in a 65° angle with CA.	Gradually narrowed to apex, not connected apically.

The pairwise genetic distance between *T.* (*V.*) *maoershanensis* sp. n. and *T.* (*V.*) *bicalcarata* was 0.028, the minimal value found when compared with the other three species, suggesting closer relationship and is in agreement with distinct morphological similarities between these two species. The distances between the two new species and the related species ranged from 0.028 to 0.091, within the range of 0.019 to 0.094, which covers all comparisons of known species inferred in the present study (Table 2). This may provide molecular evidence for the distinctiveness of these two new species.

China has a rich Tipuloidea fauna as indicated by the total numbers in the current catalogue (Oosterbroek 2016). Modern taxonomic studies on insects have been highly developed by integrating data derived from morphology, behavior, ecology, and geographic variation, which in turn is reinforced with complementary information from DNA sequences (Stoeckle 2003). Several published papers on Tipuloidea also show that the characterization of molecular data will likely contribute to our knowledge of the biodiversity and range extension of this group (Pilipenko et al. 2012, Goodman and Grady 2013, Wirta et al. 2016, Denes et al. 2016). We anticipate future intensive field collection and investigation would undoubtedly increase the species numbers and range extension of *Tipula* (*Vestiplex*) in China.

Acknowledgements

The authors wish to express sincere thanks to Dr. Pjotr Oosterbroek, University of Amsterdam, the Netherlands, for his valuable website, the Catalogue of the Crane flies of the World, from which we obtained a lot of valuable information. We are grateful to Dr. Guo-Xi Xue for his help in the collection of crane flies specimens. We are particularly grateful to Dr. Pjotr Oosterbroek and Dr. Chris Borkent for their valuable suggestions

to this paper. This study was supported by the National Natural Science Foundation of China (31300551), the Special Public Welfare Forest Foundation of State Forestry Administration (201304314), and the start-up grant for scientific research from Anqing Normal University (044-K05000130005) to Qiu-Lei Men, National Geographic Society Grant (8726-09) and National Science Council of Taiwan Grant (99WFA0100673) to Chen Young.

References

Alexander CP, Byers GW (1981) Tipulidae. In: McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM (Eds) Manual of Nearctic Diptera. Vol. 1. Biosystematics Research Institute, Ottawa, Ontario, 153–190.

Alexander CP (1935a). New or little-known Tipulidae from eastern Asia (Diptera). XXV. Philippine Journal of Science 57: 81–148.

Alexander CP (1935b) New or little-known Tipulidae from eastern Asia (Diptera). XXIV. Philippine Journal of Science 56: 525–562.

Barcenas NM, Unrun TR, Neven LG (2005) DNA Diagnostics to Identify Internal Feeders (Lepidoptera: Tortricidae) of Pome Fruits of Quarantine Importance. Journal of Economic Entomology 98(2): 299–306. https://doi.org/10.1093/jee/98.2.299

Denes A-L, Kolcsar L-P, Torok E, Keresztes L (2016) Taxonomic revision of the Carpathian endemic *Pedicia* (*Crunobia*) *staryi* species-group (Diptera, Pediciidae) based on morphology and molecular data. Zookeys 569: 81–104. https://doi.org/10.3897/zookeys.569.7458

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Frommer SI (1963) Gross morphological studies of the reproductive system in representative North American crane flies (Diptera: Tipulidae). Kansas University Science Bulletin 44: 535–625.

Goodman KR, O'Grady P (2013) Molecular phylogeny and biogeography of the Hawaiian craneflies *Dicranomyia* (Diptera: Limoniidae). PLoS ONE 8(9): e73019. https://doi.org/10.1371/journal.pone.0073019

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences of the United States of America 101(41): 14812–14817. https://doi.org/10.1073/pnas.0406166101

Hünefeld F, Beutel RG (2005) The sperm pumps of Strepsiptera and Antliophora (Hexapoda). Journal of Zoological Systematics and Evolutionary Research 43: 297–306. https://doi.org/10.1111/j.1439-0469.2005.00327.x

Johanson KA (2007) Association and description of males, females and larvae of two New Caledonian *Xanthochorema* species (Trichoptera: Hydrobiosidae) based on mitochondrial 16S and COI sequences. Entomological Science 10(2): 179–199. https://doi.org/10.1111/j.1479-8298.2007.00212.x

Khalifa A (1949) Spermatophore production in Trichoptera and some other insects. Transactions of the Entomological Society of London 100: 449–471. https://doi.org/10.1111/j.1365-2311.1949.tb01425.x

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. https://doi.org/10.1007/BF01731581

Lim P-E, Tan J, Eamsobhana P, Yong HS (2013) Distinct genetic clades of Malaysian Copera damselflies and the phylogeny of platycnemine subfamilies. Scientific Reports 3: 2977. https://doi.org/10.1038/srep02977

Men QL, Qin DZ (2011) Identification of macropterous individual of *Cixiopsis punctatus* (Hemiptera: Fulgoroidea: Tropiduchidae) based on morphological and molecular data. Entomotaxonomia 33(3): 213–219.

Men QL, Xue GX, Liu Y (2015a) A morphological study on reproductive system of *Tipula* (*Yamatotipula*) *nova* Walker (Diptera: Tipulidae). Zoological Systematics 40(3): 328–338.

Men QL, Xue GX, Yang H (2015b) Two new species of the genus *Nephrotoma* (Diptera, Tipuloidea, Tipulidae) from China with a key to species from Mainland China. ZooKeys 532: 117–136. https://doi.org/10.3897/zookeys.532.5970

Oosterbroek P (2016) Catalogue of the Craneflies of the World, (Diptera, Tipuloidea: Pediciidae, Limoniidae, Cylindrotomidae, Tipulidae). nlbif.eti.uva.nl/ccw/ [accessed 26 November 2015]

Pilipenko VE, Salmela J, Vesterinen EJ (2012) Description and DNA barcoding of *Tipula* (*Pterelachisus*) *recondita* sp. n. from the Palaearctic region (Diptera, Tipulidae). ZooKeys 192: 51–65. https://doi.org/10.3897/zookeys.192.2364

Reijnen BT, McFadden CS, Hermanlimianto YT, van Ofwegen LP (2014) A molecular and morphological exploration of the generic boundaries in the family Melithaeidae (Coelenterata: Octocorallia) and its taxonomic consequences. Molecular Phylogenetics and Evolution 70: 383–401. https://doi.org/10.1016/j.ympev.2013.09.028

Savchenko EN (1965) A new species of crane-fly (Diptera, Tipulidae) from the himalayensis-group. Dopovidi Akademii Nauk Ukrayinskoyi RSR (B) 1965: 1523–1525.

Serjeant AF, Beebee TJC (2013) Identification of *Dytiscus* larvae by cytochrome oxidase I (COI) partial sequencing. Journal of Insect Conservation 17(6): 1191–1196. https://doi.org/10.1007/s10841-013-9600-x

Stoeckle M (2003) Taxonomy, DNA, and the barcode of life. Biosciences 53: 796–797. https://doi.org/10.1641/0006-3568(2003)053[0796:TDATBC]2.0.CO;2

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882. https://doi.org/10.1093/nar/25.24.4876

Wirta H, Várkonyi G., Rasmussen C, Kaartinen R, Schmidt NM, Hebert PDN, Barták M, Blagoev G, Disney H, Ertl S, Gjelstrup P, Gwiazdowicz DJ, Huldén L, Ilmonen J, Jakovlev J, Jaschhof M, Kahanpää J, Kankaanpää T, Krogh PH, Labbee R, Lettner C, Michelsen V, Nielsen SA, Nielsen TR, Paasivirta L, Pedersen S, Pohjoismäki J, Salmela J, Vilkamaa P,

Väre H, von Tschirnhaus M, Roslin T (2016) Establishing a community-wide DNA barcode library as a new tool for arctic research. Molecular Ecology Resources 16(3): 809–822. https://doi.org/10.1111/1755-0998.12489

Yang ZF, Landry JF, Handfield L, Zhang YL, Solis MA, Handfield D, Scholtens BG, Mutanen M, Nuss M, Hebert PDN (2012) DNA barcoding and morphology reveal three cryptic species of *Anania* (Lepidoptera: Crambidae: Pyraustinae) in North America, all distinct from their European counterpart. Systematic Entomology 37(4): 686–705. https://doi.org/10.1111/j.1365-3113.2012.00637.x